

JAHA submission, accepted version 25th Sept 2019**Remote Ischaemic Conditioning After Stroke Trial 2: a phase IIb randomised controlled trial in hyperacute stroke**

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Abstract

Background

Repeated episodes of limb ischaemia and reperfusion (remote ischaemic conditioning, RIC) may protect the brain from ischaemic reperfusion injury.

Methods and Results

We performed a phase IIb blinded dose-escalation sham-controlled trial in patients with hyperacute stroke, randomised 1:1 to receive RIC (four 5-minute cycles) or sham to the non-paretic upper limb, in 3 blocks of increasing dose, starting within 6 hours of ictus. The primary outcome was trial feasibility (recruitment, attrition). Secondary outcomes included adherence, tolerability, safety (serious adverse events [SAE]), plasma biomarkers at day 1 and 4 (S100- β protein, matrix metalloproteinase-9 (MMP-9) and neurone specific enolase (NSE)) and functional outcome. Sixty participants were recruited from two centres (3/month) with no loss to follow-up: time to randomisation 4hr 5min (standard deviation 72min), age 72 (12), male 60%, blood pressure 154/80mmHg (25/12), NIHSS 8.4 (6.9) and 55% thrombolysed. RIC was well tolerated with adherence not differing between RIC and sham, falling in both groups on day 3 ($p=0.001$, repeated measures ANOVA) due to discharge or transfer. S100 β increased in the sham (mean rise 111pg/ml (302), $p=0.041$, repeated measures ANCOVA) but not RIC group. There were no differences in MMP-9, NSE, number with SAE (RIC 10 v sham 10, $p=0.81$), deaths (2 v 4, $p=0.36$) or modified Rankin score (2 [interquartile range 1-4], 2 [1-3], $p=0.85$).

Conclusions

RIC in hyperacute stroke is feasible when given twice daily for two days and appears safe in a small population with hyperacute stroke. A larger phase III trial is warranted.

ClinicalTrials.gov Identifier: NCT02779712

Key words: stroke, remote ischaemic conditioning, feasibility, randomised controlled trial

Clinical Perspective

- This is the first randomised controlled trial in hyperacute ischaemic stroke testing an increasing dose of remote ischaemic conditioning; repeated dosing until day two was feasible in terms of adherence, and the dosing regimen for larger RIC trials should consider this alongside local patient pathways.
- Beneficial clinical signals exist from several small pilot and proof-of-concept studies using RIC after stroke and these findings warrant further testing in a well-designed, larger phase III trial.

BACKGROUND

Remote ischaemic conditioning (RIC) uses repeated cycles of transient limb ischaemia and reperfusion to induce organ protection from ischaemic reperfusion injury and tolerance to subsequent ischaemic events. The mechanisms underlying RIC are not fully understood but have been attributed to neuro-humoral pathways linking the pre-conditioned tissue to the brain, resulting in early and late windows of protection.^{1, 2}

Experimentally, RIC applied before (pre-conditioning), during (per-conditioning) and after (post-conditioning) an ischaemic stroke decreases cerebral inflammation and oedema, and reduces apoptosis in the ischaemic penumbra through inhibition of the mitochondrial permeability transition pore (MPTP).³⁻⁵ Administration of a protein synthesis inhibitor, afferent nerve blocker or K_{ATP} channel antagonist attenuates the neuroprotective effects of RIC.^{4, 6} In meta-analysis of pre-clinical stroke, remote pre- per- and post-conditioning reduced infarct volume by 35%, was effective in permanent and transient models of ischaemia, and improved neurological outcome.⁷

Five proof-of-concept randomised clinical trials in stroke and RIC have been published: in populations prior to carotid stenting (pre-conditioning),⁸ in acute ischaemic stroke (per-conditioning),^{9, 10} and after ischaemic stroke caused by intracranial stenosis (post-conditioning).^{11, 12} In the Remote Ischaemic Conditioning After Stroke Trial (RECAST-1), we demonstrated excellent intervention tolerability in patients with acute stroke;¹⁰ although limited by a small sample size, there was also a significant decrease in National Institutes for Health Stroke Scale (NIHSS) score at day 90, and augmentation of neuroprotective proteins, plasma HSP27 and phosphorylated HSP27, in the RIC group.¹³

In the current study, we aimed to demonstrate feasibility and safety of increasing doses of remote ischaemic per-conditioning in patients presenting to hospital with hyperacute stroke.

METHODS

Trial Design

The Remote ischaemic Conditioning After Stroke Trial 2, RECAST-2, was a two-centre, feasibility, dose-escalation, outcome blinded, randomised placebo-controlled trial. The trial was conducted in accordance with the Declaration of Helsinki and the International Conference of Harmonisation of Good Clinical Practice (ICH-GCP), sponsored by the University of Nottingham (UK), received authorisation from the Local Research Ethics Committee (LREC, West Midlands, 15th April 2016) and was a registered clinical trial (NCT02779712).

Subjects

Adults ≥ 18 years were invited to participate if they had a clinical stroke with onset in the last 6 hours. Exclusion criteria were premorbid dependency (modified Rankin Scale >3), dementia, Glasgow Coma Score <8 , malignancy, pregnancy and significant co-morbidity. We did not mandate that baseline neuroimaging (as part of standard care) was required before randomisation, but in practice, the brain scan and approaching the participant for the trial occurred in parallel, with the result available prior to trial inclusion. The trial recruited patients from University Hospitals of Derby and Burton (UHDB) NHS Foundation Trust and Nottingham University Hospitals (NUH) NHS Trust in the UK between August 2016 and April 2018. UHDB and NUH receive approximately 900 and 1300 strokes per year respectively. Consent was obtained by the research practitioner from each patient or legal representative if the patient was unable to consent. Clinical and safety assessments were performed at baseline (pre-randomisation), day 4 (face-to-face) and day 90 (telephone).

Intervention

RIC was performed by trained trial staff immediately after randomisation and included 4 cycles of intermittent limb ischaemia: alternating 5 minutes inflation (20 mmHg above systolic BP) followed by 5 minutes deflation of a standard upper arm blood pressure cuff in the non-paretic arm. The control group received a sham procedure mimicking the intervention protocol but cuff inflation only reached 30 mmHg. The intervention was performed manually using a standard BP cuff. Preclinically, repeated RIC cycles is more effective than a single set of cycles,¹⁴ hence we increased the dose in three phases: the first 20 participants received one 'dose' of RIC/sham, i.e. 4 cycles of cuff inflation and deflation;

participants 21-40 received a second dose (4-cycles of RIC/sham) one hour after the first dose; and the final 20 participants (41-60) were also administered twice daily dosing starting the following morning up to and including day 4 (total 8 doses). Delivery time of each cycle was recorded (seconds) and reasons for discontinuation.

Primary Outcome

The primary outcome was trial feasibility describing recruitment rate, time to recruitment, number recruited per centre, and attrition to follow up.

Secondary Outcomes

Tolerability

Tolerability of increasing doses of RIC: duration cuff tolerated, number of cycles, adherence to RIC and reasons for poor adherence.

Clinical measures and safety

Clinical secondary outcomes included safety: vascular events (recurrent stroke, myocardial infarction, limb ischaemia and venous thrombo-embolism), death, neurological deterioration (ND, increase in NIHSS ≥ 4 points), neurovascular limb damage and tissue injury. Comparison of serious adverse events (SAE) by treatment with thrombolysis provided a further assessment of safety in this subgroup. Function was assessed at day 90 by telephone interview blinded to treatment allocation: dependency (mRS),¹⁵ disability (Barthel Index),¹⁶ Zung depression scale,¹⁷ quality of life (EQ-5D) and cognition (TICS-M).¹⁸

Laboratory measures

Immediately prior to treatment and on day 4 (± 1), blood samples were collected for (i) surrogate markers of brain injury, which might be attenuated if RIC improves ischaemic reperfusion injury (plasma S100 β protein, matrix metalloproteinase (MMP-9), neurone specific enolase (NSE), by multiplex technology, Merck Millipore Ltd, UK); S-100 β , NSE and MMP-9 also act as surrogate markers of infarct volume and prognosis in acute ischemic stroke.^{19, 20} (ii) Heat shock protein-27 (HSP-27, DouSet ELISA, R&D Systems, Abingdon, UK), is a biomarker implicated in the mechanisms of ischaemic conditioning¹⁰ and is neuroprotective in experimental stroke.¹³ Assays and analysis of data were performed blinded to treatment allocation.

Sample Size

No formal power calculation was performed for this feasibility study. Considering resources, competing trials and time, recruiting 60 participants from two centres was deemed an appropriate number, at an anticipated rate of recruitment of 1.5 recruits per centre per month, to inform a larger trial on application of RIC in the hyperacute setting in terms of trial feasibility and increasing RIC dose.

Randomisation and blinding

Participants were recruited using web-based randomisation with computerised minimisation distributing the patients on a 1:1 ratio into RIC or sham groups. Minimisation variables were age (≥ 70), sex (male), NIHSS (≥ 10) and systolic BP (≥ 160 mmHg). The research practitioner delivering the intervention could not be blinded. Adjudicated serious adverse events and outcomes, day 90 interview, laboratory measures and statistical analyses were performed blinded to treatment allocation.

Statistical Methods

Baseline characteristics and functional outcomes of RIC and control groups were compared using Chi-squared or Fisher's Exact test for binary data; continuous data are compared using t-test or Mann-Whitney U test; recurrent clinical events were compared using hazard ratios and univariate Cox-regression analyses (SPSS Statistics version 24). Additionally, day 90 mRS was compared using ordinal logistic regression. Repeated measures ANOVA with no co-variate adjustment compared adherence to treatment between groups. Repeated measures ANCOVA, adjusting for baseline NIHSS, compared plasma biomarkers taken on day 1 and day 4, with further adjustment using Sidak's multiple comparisons test (SPSS Statistics version 24 and Prism 7 for Mac OS X version 7.0c). Associations between S100 β and functional outcome were tested using Pearson's correlation coefficient. Subgroup analyses were not performed at a dose level since numbers were considered too small. Data in the figures are mean values \pm standard deviation (SD) unless otherwise stated. Statistical significance was taken at $p < 0.05$.

Data Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

RESULTS

Subjects

The trial commenced in August 2016, completed follow up in August 2018 and enrolled 60 participants (31 and 29 in the RIC and sham groups respectively, 20 per dose block, Figure 1). The mean age of all participants was 72 years (SD 12), 60% male, mean blood pressure (BP) 154/80 (SD 25/12) mmHg and median NIHSS 6 [interquartile range, IQR 3-11]. There were no baseline statistical differences between groups for age, sex and baseline stroke severity (Table 1). The RIC group were randomised later (254 versus 195 minutes, $p=0.003$), contained more participants with diabetes (33% versus 7%, $p=0.02$) and had a lower mean systolic BP (146 versus 162 mmHg, $p=0.01$). The final diagnosis was ischaemic stroke in 55 (92%), transient ischaemic attack in 4 (7%) and functional disorder in 1 (2%).

Trial Feasibility

Recruitment rate averaged 1.5 participants per centre per month ($n=20$ centre 1, $n=40$ centre 2). The main reasons for exclusion included presentation greater than 6 hours from onset of symptoms (42.5%), presenting outside of working hours (13.4%) and non-stroke diagnoses (12.5%) (Supplementary Table I). The median time to randomisation was 255 minutes [IQR 186-298] with 33 (55%) receiving thrombolysis. There were no losses to follow-up. The sham appeared feasible since when asked at day 90 which intervention they received, 56 (93%) did not know, 2 (4%) were incorrect and 2 (4%) correct.

Compliance

RIC was well tolerated with no statistical differences between RIC and sham regarding duration of cuff inflation (Figure 2). Adherence was high in the first 2 days but there was a significant fall on day 3 (dose 5) in RIC and sham groups to 40% and 43% respectively ($p=0.001$, repeated measures ANOVA), with no between group differences ($p=0.64$) secondary to either early discharge or the participant moving to another rehabilitation setting. In the first 48 hours, procedure intolerance in the RIC group (cuff pressure intolerance, headache, agitation) leading to incomplete treatment of 4 cycles occurred in 5 of 62 (8%) offered doses (Supplementary Table II).

Safety and clinical outcomes

There was no difference in the number of participants with a SAE (Table 2 and Supplementary Table III) and no episodes of limb ischaemia or injury. Mortality was 10%

with 4 deaths in the sham group (n=1 extension/recurrent ischaemic stroke, n=1 haemorrhagic transformation of infarction (HTI), n=1 early ND, n=1 gradual decline) and 2 in the RIC group (n=1 HTI, n=1 malignancy). Extension and recurrent ischaemic stroke were more frequent in the sham group (6 versus 2 events, unadjusted HR 0.28, 95% confidence interval CI 0.06 - 1.37, p=0.12). 83% of recurrent cerebrovascular events occurred in the first 48 hours. RIC appeared safely administered in the thrombolysed cohort with no differences in SAEs between groups (Supplementary Table IV).

Laboratory Measures

Plasma S100 β increased significantly in the sham group from day 1 to day 4, mean difference (MD) 111 pg/ml (95% CI 5.6 – 216, p=0.041, repeated measures ANOVA, adjusted for baseline NIHSS, Figure 3). No differences in plasma S100 β were present in the RIC group between days 1 and 4 (MD 27.5 pg/ml 95% CI -14.5 – 69.5, p=0.187) nor were there significant differences between groups at day 4 (adjusted p=0.35). Day 4 Plasma S100 β correlated significantly with baseline NIHSS (Pearson's correlation coefficient, r=0.561, p<0.001) and day 90 mRS (r=0.41, p=0.006). MMP-9 concentration was non-significantly higher in the sham group compared to RIC (change from baseline, MD 15.3 ng/ml (95% CI -2.6 – 33.2), p=0.09). There were no differences between groups with respect to NSE. Heat shock protein 27 assays proved unreliable and data is not presented.

Functional outcomes

There were no significant differences between groups with respect to functional outcomes, though the trial was not large enough to detect these (Table 3 and Figure 4). Telephone data collection at day 90 was feasible for measures of dependency (mRS), disability (BI), mood (Zung), cognition (TICS-M) and quality of life (EuroQoL), similar to other large trials.²¹

DISCUSSION

The Remote ischaemic Conditioning After Stroke Trial 2 has demonstrated the feasibility of conducting a randomised controlled trial of remote ischaemic per-conditioning in hyperacute stroke across two centres in terms of recruitment, intervention delivery, attrition, compliance of increasing dose to day two, and use of an effective sham.

RECAST-2 is the first stroke and RIC trial to evaluate alternative dosing strategies. Overall, the optimal dosing and method of application of RIC in stroke remains unclear. There is noticeable heterogeneity in completed and ongoing clinical trials ranging from daily administration using both arms in post-conditioning secondary prevention studies (cuff pressure to 200 mmHg),^{11, 12} to single lower limb application using cuff pressures 120 mmHg above the systolic BP in acute ischaemic stroke.²² Strategies appear to be based on the population studied rather than from information provided by pre-clinical data. Importantly, an experimental dose-finding study in post-conditioned stroke rats determined that 3 cycles of 5min/5min limb ischaemia and reperfusion was more effective than 15sec/15sec and 8min/8min.⁴ Previous trials have delivered RIC daily for up to 300 days,^{11, 12} initiated in the subacute phase post stroke; it is therefore feasible to deliver RIC for a prolonged period using an automated machine. We chose the maximum dose to stop at day 4 since this covers the hyperacute phase and prolonged effects of the treatment are anticipated.²³ We also expected it would not be possible to administer RIC using a manual BP cuff for longer than this, which proved to be the case: in RECAST-2, repeated dosing until day two was feasible in terms of adherence, and the dosing regimen for larger RIC trials should consider this alongside local patient pathways. The main reason for treatment discontinuation was not cuff pressure intolerance but transfer of the participant to a different setting or discharge home.

The absence of any serious adverse events relating to limb ischaemia or injury, especially in the thrombolysed cohort, is reassuring. The safety of RIC in hyperacute stroke, however, requires further evaluation since this is a small population. RIC has potential anti-platelet effects,²⁴ which may be beneficial in ischaemic stroke, but could exacerbate haemorrhagic transformation of infarction or lead to deterioration if administered in intracerebral haemorrhage prior to confirmation of the diagnosis; one pre-hospital RIC trial, however, reported no clinical deterioration in thirty-seven participants with primary intracerebral haemorrhage.⁹

The majority of recurrent cerebrovascular events occurred within the first 48 hours, reflecting early ischaemic reperfusion injury, which can manifest clinically as recurrent ischaemia, haemorrhagic transformation of infarction, cerebral oedema and expansion of the original infarct. The trial was not powered to detect reductions in these events, but we observed tendency in favour of RIC towards reduced risk of recurrent fatal and non-fatal stroke. In addition, there are biochemical signals of efficacy evidenced by increased plasma biomarkers of brain injury (S100 β) in the placebo group not seen in the RIC group. S100 β is a recognised surrogate marker of infarct volume and functional outcome,¹⁹ and in this study correlated significantly with baseline stroke severity and day 90 mRS.

It is recognised that RIC leads to an immediate period of ischaemic tolerance lasting 1-2 hours, followed by a second window of protection (SWOP) 12-24 hours later, lasting 48-72 hours.²⁵ Pre-clinically, alteplase combined with RIC has an additive effect²⁶ and a single dose of RIC can have long-lasting protective effects for up to 6 days.²³ Further, the time-window of RIC application in experimental models extends up to 6 hours post-ictus²⁷ and combining per- and post-conditioning may tackle both early and late phases of ischaemic reperfusion injury. Per-conditioning was more effective at reducing infarct volume than a pre-conditioning stimulus in one study²⁸ but this difference is not borne out in meta-analysis of experimental data.⁷ Since strokes are difficult to predict, per-conditioning is a viable strategy in acute ischaemia, whereas pre-conditioning maybe more suited towards high-risk populations, for example, before carotid intervention,⁸ or after a transient ischaemic attack.

A recent Cochrane Review exploring RIC for preventing and treating ischaemic stroke has highlighted the paucity of published randomised clinical trials in this area.²⁹ Interestingly, recurrence in ischaemic stroke (by end of trial) was significantly reduced.^{8, 11, 12, 29} In updating the meta-analysis with RECAST-1,¹⁰ RECAST-2 and Che 2019,³⁰ and organising groups into pre- per- and post-conditioning trials, RIC significantly reduces the composite outcome of recurrent vascular events, an OR 0.27 (95% CI 0.13-0.59), Figure 5.^{8, 10-12, 30} This is consistent with secondary analyses in the cardiac literature (RIC and acute myocardial infarction) where recurrent cardiovascular and cerebrovascular events were reduced by half.³¹ It is not intuitive that brief periods of RIC can lead to protection from vascular events at much later time points (and repeated doses may be required) but the finding deserves further exploration in clinical trials.

RECAST-2 was a high-quality randomised trial strengthened by assessment of multiple doses, concealment of allocation and blinded outcome assessments. Limitations include the inability to blind the investigator performing the intervention, potentially introducing bias into RIC/sham compliance. Second, a small sample size, which is sufficient to answer questions of feasibility, introduces risk that other findings may be due to chance, especially since there was an imbalance in systolic blood pressure and diabetes between groups at baseline; a larger randomised trial is needed to further evaluate efficacy and safety. Third, no participants undergoing mechanical thrombectomy (MT) were included (due to the need to deliver RIC manually and logistics of transfer to another site); we are unable to comment on safety of RIC in MT but RIC seems feasible and safe in an observational study of MT.³² Finally, due to a limited budget, we did not perform any mechanistic neuroimaging studies that determine recanalization or reperfusion rates; however, whilst use of RIC in acute ischaemic stroke seems most likely to benefit those with ischaemic reperfusion injury, there is suggestion that in patients with persisting occlusion, RIC may still reduce infarct risk.⁹

In summary, RIC in hyperacute ischaemic stroke is feasible, appears safe and can be administered in repeated doses reliably for two days. It is an attractive prospect since it bears low cost and would be simple to administer. A larger phase III trial is warranted.

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Disclosures

There are no conflicts of interest to declare. PB is Stroke Association Professor of Stroke Medicine, and is a NIHR Senior Investigator.

Author contributions

TE designed the trial, analysed the data and wrote the manuscript. AH and BJ screened for participants, acquired the data and helped revise the manuscript. SOS processed and analysed blood biomarkers, and revised the manuscript. NS and PB contributed to trial design and management, and revised the manuscript.

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Table 1. Baseline characteristics

Characteristic	RIC	Sham
Number	31	29
Age, years (SD) [†]	70.9 (13.4)	73.7 (10.2)
Male (%) [†]	21 (70)	15 (50.0)
Blood pressure, mmHg		
Systolic [†]	146 (24)	162 (23)
Diastolic	78 (12)	83 (11)
Heart rate	77 (13)	80 (18)
Admission ECG in AF	11 (36.7)	12 (40.0)
NIHSS [†]	6 [3-9]	7 [3-12]
GCS	15 [14-15]	15 [14-15]
Premorbid mRS	0 [0-2]	0 [0-1]
Stroke to randomisation [minutes]	254 [254-343]	199 [149-261]
Admission to randomisation [minutes]	195 [174-277]	93 [66-168]
Thrombolysed	16 (51.6)	17 (58.6)
Mechanical thrombectomy	0 (0)	0 (0)
Final diagnosis (%) [*]		
Ischaemic stroke	28 (90.3)	27 (93.1)
TIA	2 (6.5)	2 (6.9)
Haemorrhagic stroke	0 (0)	0 (0)
Clinical syndrome (%)		
Total anterior circulation	6 (20.0)	5 (16.7)
Partial anterior circulation	9 (30.0)	14 (46.7)
Lacunar	9 (30.0)	8 (26.7)
Posterior circulation	4 (13.3)	0 (0.0)
Past Medical History (%)		
Hypertension	14 (46.7)	11 (36.7)
Diabetes mellitus	10 (33.3)	2 (6.7)
Known AF	12 (40.0)	10 (33.3)
Hyperlipidaemia	14 (46.7)	9 (30.0)
Stroke	9 (30.0)	5 (16.7)
TIA	6 (20.0)	2 (6.7)
Ischaemic Heart Disease	5 (16.7)	4 (13.3)
Peripheral Vascular Disease	1 (3.3)	1(3.3)

Data presented are mean values (standard deviation), median [interquartile range] or number (percentage). AF, atrial fibrillation; NIHSS, National Institute of Health Stroke Scale; mRS, modified Rankin scale; TIA, transient ischaemic attack; [†] minimisation variables, ^{*} one participant diagnosed with functional disorder in the RIC group.

Table 2

Summary of secondary clinical outcomes and serious adverse events

Serious Adverse Event	RIC (n=31)	Sham (n=29)	HR (95% CI)	p
No with SAE				
Any SAE	10 (32.3)	10 (34.5)	0.81 (0.33-1.96)	0.81
Fatal	2 (6.5)	4 (13.8)	0.46 (0.8-2.5)	0.36
All stroke and ND*				
Extension/recurrent ischaemic stroke	2 (6.5)	6 (20.7)	0.28 (0.06-1.37)	0.12
Symptomatic HTI	2 (6.5)	1 (3.4)	1.85 (0.17-20.38)	0.62
Early ND	1 (3.2)	0 (0.0)	-	-
Seizure	0 (0.0)	1 (3.4)	-	-
TIA	1 (3.2)	0 (0.0)	-	-
MI	0 (0.0)	0 (0.0)	-	-
VTE				
PE	0 (0.0)	1 (3.4)	-	-
DVT	0 (0.0)	0 (0.0)	-	-

SAE, serious adverse event, ND neurological deterioration, HTI haemorrhagic transformation of infarction; TIA transient ischaemic attack; MI, myocardial infarction; VTE, venous thrombo-embolism; PE, pulmonary embolism; DVT, deep vein thrombosis. Analyses performed using unadjusted Cox-regression.

*1 participant in the sham group had ND and HTI; 1 in the RIC group had HTI and recurrent stroke (only the first event is counted in regression analyses).

Table 3

Functional outcome by group at day 4 and day 90

Functional measure	RIC	Sham	p
Day 4 *	n=30	n=24	
NIHSS	6.4 (9.4)	9.5 (12.8)	0.30
Change NIHSS	-3.0 (3.8)	-1.4 (6.3)	0.35
Day 90	n=31	n=29	
Modified Rankin Scale (/6)			
Median	2 [1, 4]	2 [1, 3]	0.85
mRS 3-6 (%)	12 (40)	14 (46.6)	0.46
Barthel Index (/100)	100 [65, 100]	100 [57.5, 100]	0.89
Zung depression score [∂]	46.25 [33.75, 53.75]	42.5 [37.5, 52.5]	0.94
EuroQoL HUI [∂]	0.514 (0.377)	0.482 (0.393)	0.77
EuroQoL VAS [∂]	70.8 (23.0)	69.8 (19.2)	0.87
TICS-M [∂]	23 [20, 25]	23.5 [21, 27]	0.89

NIHSS, Nation Institute of Health Stroke Scale; mRS, modified Rankin scale, HUI health utility index; VAS visual analogue scale

Data are mean (SD), median [IQR] or number (%). Imputed value for death: BI -5; NIHSS 42

* Day 4 NIHSS - Sham n=24, RIC n=30 (data missing due to early discharge or refused)

Analysed by independent t-test, Mann-Whitney U test or Chi square test as appropriate.

[∂] N for EuroQoL HUI: 24(sham) / 28(RIC), EuroQoL VAS 22/24, Zung 17/16, TICS-M 14/14. N reduced by: (i) carers answering on behalf of participants who could not respond (n=17), (ii) refused to answer questions on mood and cognition, (iii) death (n=6).

Figure Legends

Figure 1. Trial Flow.

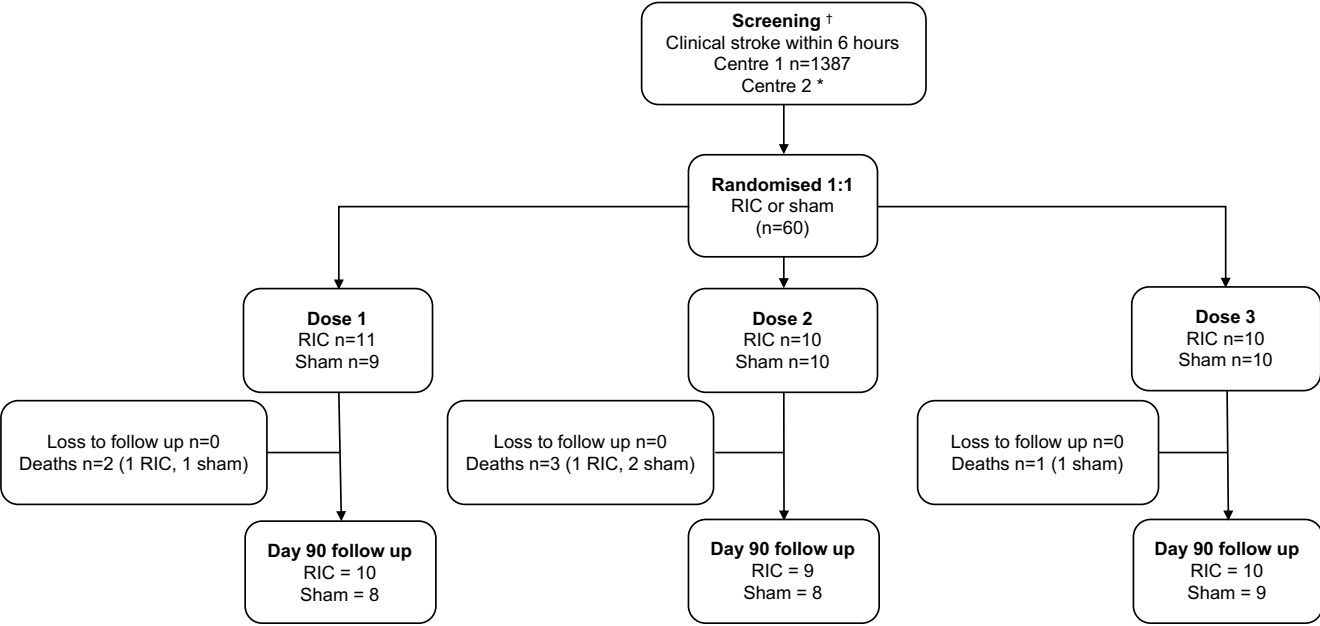
Figure 2. Adherence to RIC (remote ischaemic conditioning) or sham by dose number and mean total duration of limb ischaemia (seconds \pm standard deviation). Maximum length of cuff inflation is 300 seconds per dose (4x 5 minutes/cycle). Compared to dose 1, there is a significant fall in adherence over time from Day 3 (*p=0.001, **p<0.001 repeated measures ANOVA), with no between group differences (p=0.64). 'n' sham/RIC = dose 1 29/31; dose 2 19/21; doses 3-8 10/10.

Figure 3. Plasma S100 β (A), matrix metalloproteinase-9 (MMP-9, B) and neurone specific enolase (NSE, C) on days 1 and 4 by treatment group. S100 β levels increase by day 4 in the sham group from 34.5 pg/ml (SD 37.8) to 145.6 pg/ml (309.1), mean difference 111 pg/ml (95% CI 5.6 - 216), p=0.041*. There were no significant between group differences at day 4. Analysis by repeated measures ANCOVA, Sidak's correction for multiple comparisons and adjusted for baseline stroke severity. RIC = remote ischaemic conditioning.

Figure 4. Day 90 modified Rankin (mRS) score by treatment group. Unadjusted common odds ratios (cOR) and 95% confidence intervals (CI) comparing groups are analysed by ordinal logistic regression. There was no significant interaction when treatment*thrombolysis was introduced into the model. The line demarcates dichotomy at functional independence, a modified Rankin score (mRS) of ≤ 2 . RIC = remote ischaemic conditioning.

Figure 5. Recurrent vascular events (non-fatal and fatal stroke, non-fatal and fatal myocardial infarction) in randomised controlled trials assessing remote ischaemic conditioning (RIC) in stroke. ref = reference number.

Figure 1



†Supplementary Table I details full screening breakdown
*Data not available from Centre 2

Figure 2

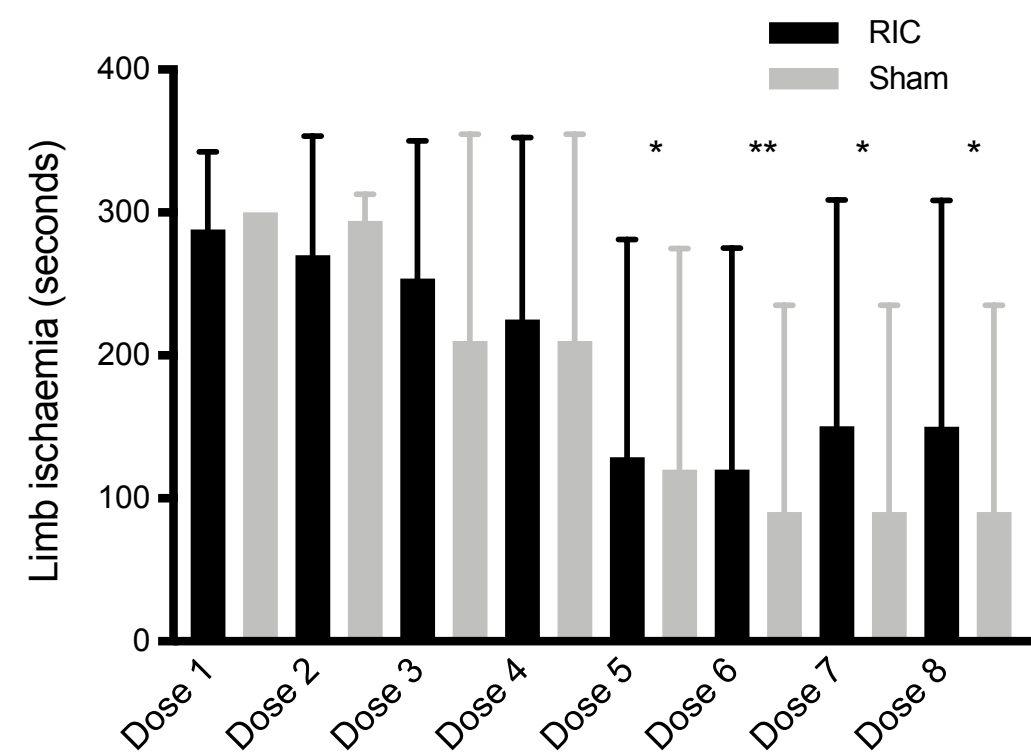


Figure 3

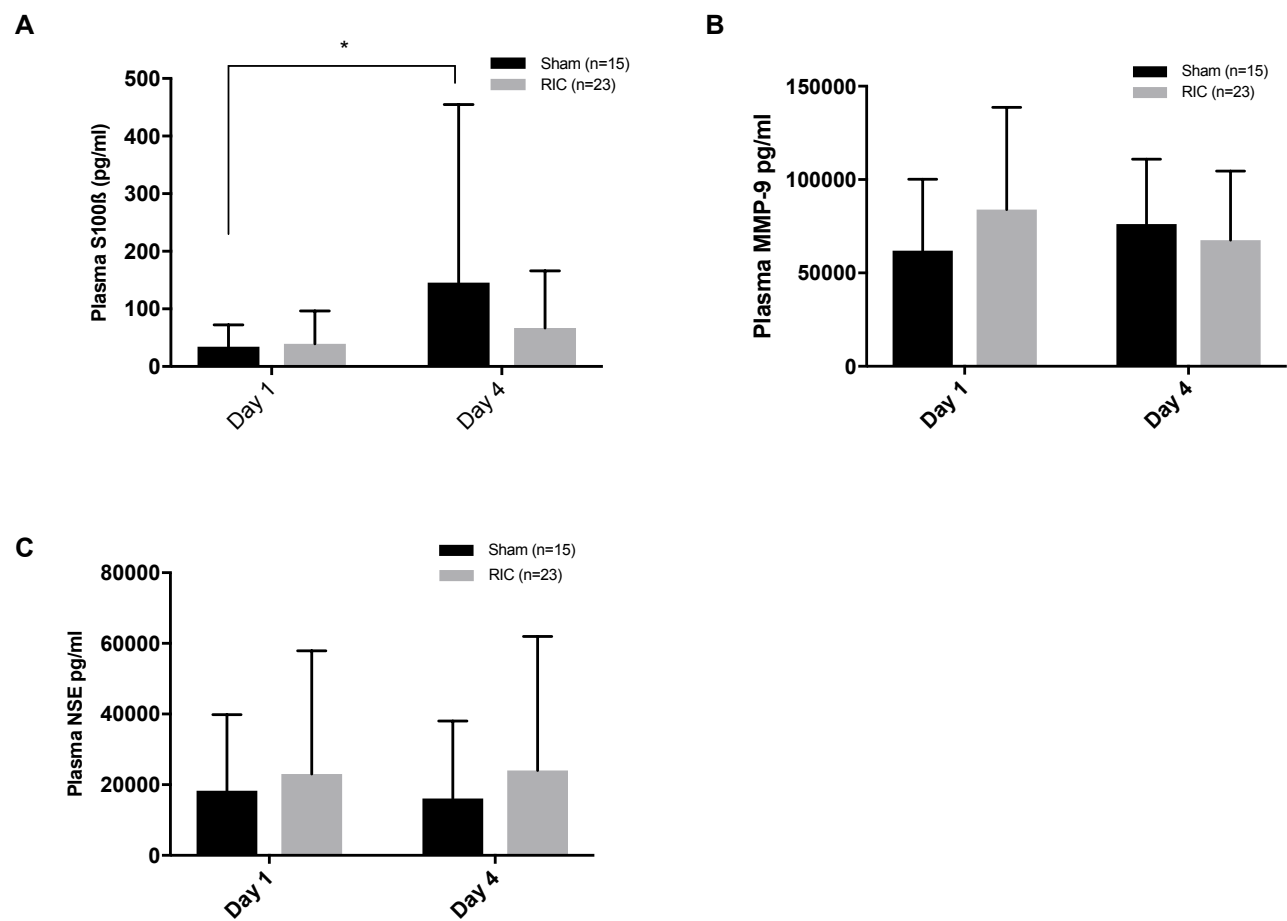


Figure 4

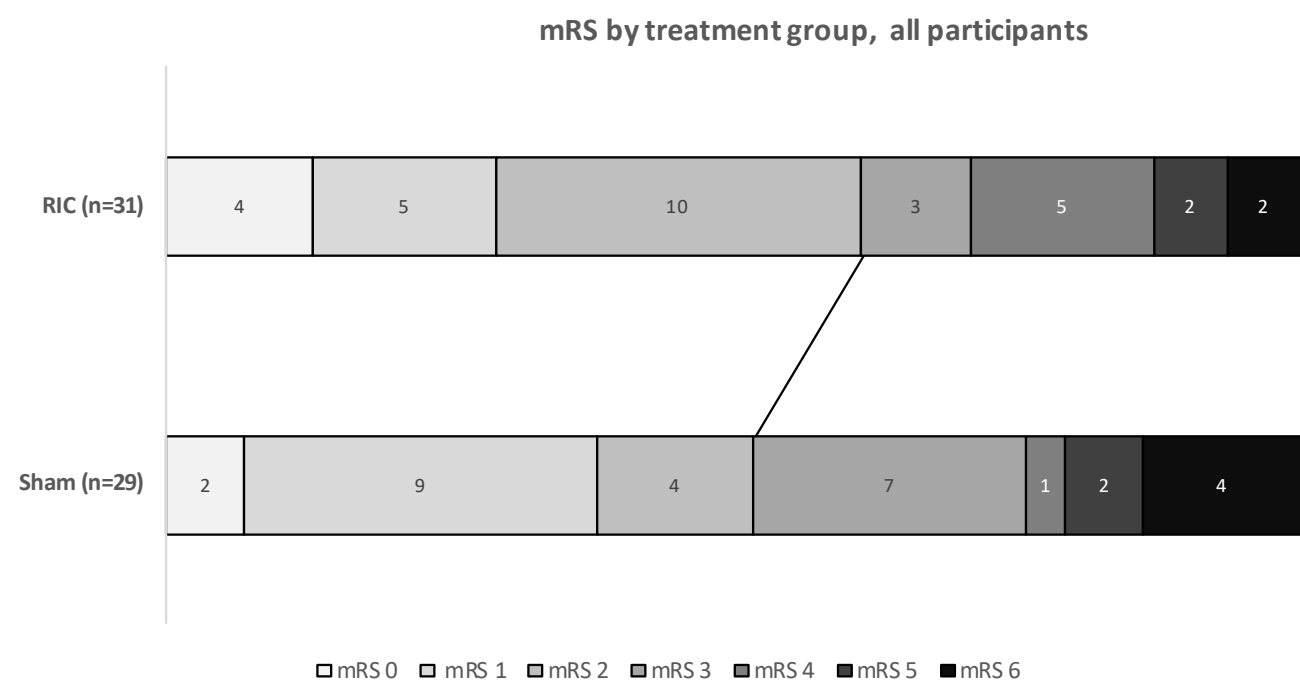
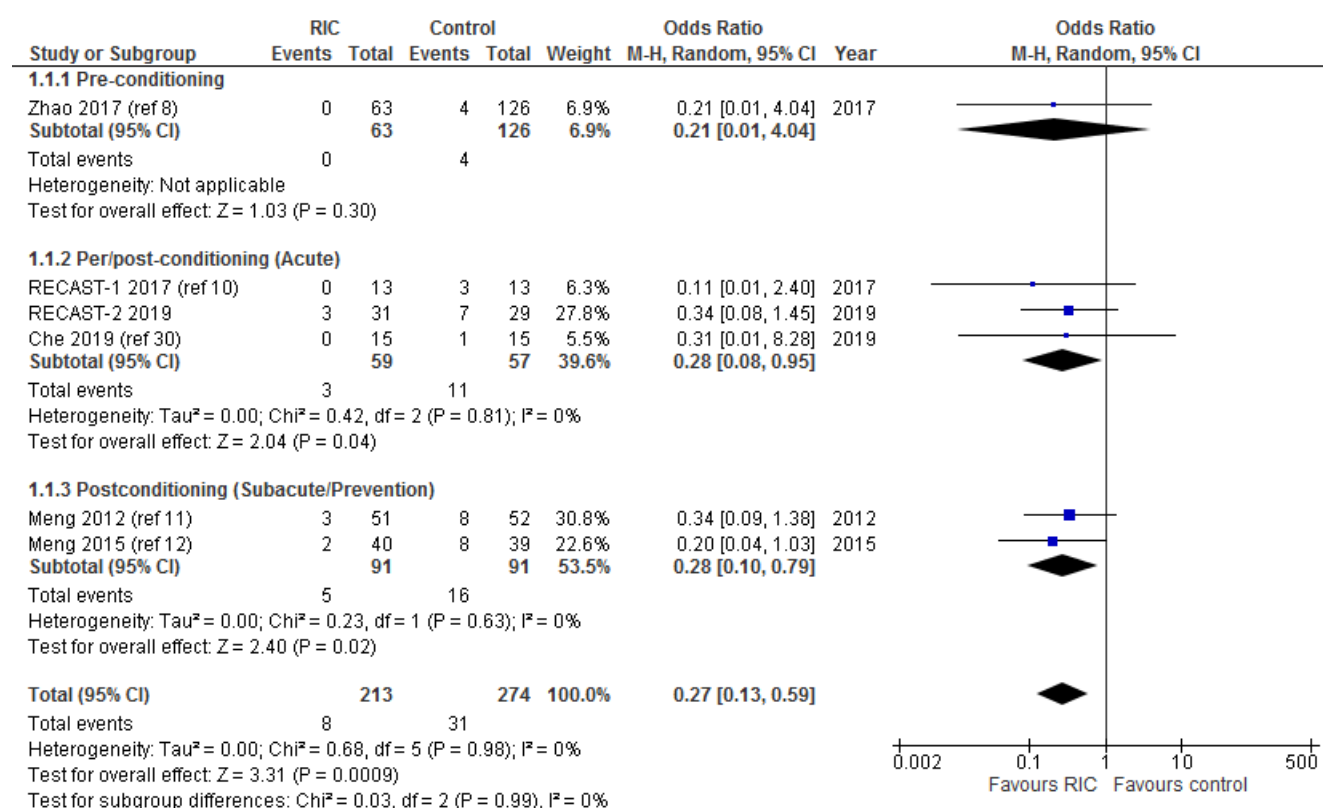


Figure 5

Supplementary Table I

Reasons for exclusion from centre 1. (Data not available from centre 2, which were discarded before analysis due to changes in European law on data protection (General Data Protection Regulation (GDPR), see <https://eugdpr.org>)

Reason for exclusion	N = 1387	(%)
Greater than 6 hours since onset of symptoms	575	41.5
Premorbid dependency mRS \geq 4	12	0.9
Dementia	34	2.5
Coma – GCS $<$ 8	3	0.2
Malignancy or significant co-morbidity thought to limit life expectancy	12	0.9
Blood glucose $<$ 3.5mmol/L	0	0.0
Pregnancy	0	0.0
Out of hours	186	13.4
Non stroke	174	12.5
No-one to assent	8	0.6
Trial on hold	9	0.6
Poor prognosis/ Died	11	0.8
Recruited	20	1.4
Refused	7	0.5
Out of area	1	0.1
No English	2	0.1
Competing trial	13	0.9
Anaphylactic reaction to thrombolysis	2	0.1
Resolved minor stroke	8	0.6
TIA	119	8.6
ICH	170	12.3
Thrombectomy	3	0.2
Seizures/ vomiting at presentation	1	0.1
Researcher unavailable (e.g. annual leave, training, sick leave)	17	1.2

Supplementary Table II

Reasons for non-compliance

RIC group	
Dose 1	n=1 refused all cycles [†]
Dose 2	n=1 refused all cycles [†] , n=4 reduced compliance (1=cuff pressure, 1=headache, 2=cannula)
Dose 3	n=1 weekend (no researcher available to administer intervention), n=1 relative refusal, n=1 cuff pressure
Dose 4	n=1 agitated, refused remaining doses; n=1 weekend; n=1 relative refused remaining doses; n=1 cuff pressure
Dose 5	n=2 weekend, n=1 relative refusal, n=1 felt unwell on cuff release, remainder discharged
Dose 6	n=2 weekend, n=1 refused (previously felt unwell with cuff release), n=1 relative refused, remainder discharged
Dose 7	n=1 refusal, n=1 relative refusal, remainder discharged
Dose 8	n=1 refusal, n=1 relative refusal, remainder discharged
Sham	
Dose 2	n=1 deteriorated during treatment, n=1 relative refused all doses after dose 1, n=1 weekend
Doses 3&4	n=1 weekend (no researcher available to administer intervention)
[†] one participant refused doses 1 and 2 but was fully compliant doses 3-8 Early discharge explains the remainder of non-compliance in the sham group	

Supplementary Table III. Serious adverse events

Treatment Group	Trial number	Thrombo - lysis	Time post randomisation (days d, hours hr, minutes m)	Adjudicated Diagnosis	Day of death	Relationship
Sham	11	Y	0 d, 5 hr, 58 m	Extension/recurrent ischaemic stroke	3	Improbable
	11	Y	0 d, 6 hr, 33 m	Pneumonia		Improbable
	13	N	0 d, 15 hr, 7 m	Extension/recurrent ischaemic stroke		Improbable
	13	N	0 d, 0 hr, 2 m	Haematemesis	6	Improbable
	13	N	3 d, 12 hr, 17 m	Pulmonary embolism		Improbable
	15	N	0 d, 9 hr, 54 m	Extension/recurrent ischaemic stroke		Improbable
	23	N	0 d, 0 hr, 36 m	Extension/recurrent ischaemic stroke	4	Improbable
	23	N	1 d, 21 hr, 56 m	Symptomatic Haemorrhagic transformation of infarct		Possible
	28	Y	0 d, 3 hr, 29 m	Seizure / convulsions		Improbable
	28	Y	0 d, 0 hr, 1 m	Early neurological deterioration	47	Improbable
		Y	0 d, 22 hr, 19 m	Asymptomatic Haemorrhagic transformation of infarct		Possible
	41	Y	1 d, 22 hr, 20 m	Pneumonia		Improbable
	38	N	15 d, 22 hr, 39 m	Traumatic rectus sheath haematoma	47	Improbable
	38	N	6 d, 0 hr, 30 m	Urinary tract infection		Improbable
	41	Y	12 d, 23 hr, 11 m	Pneumonia		Improbable
	41	Y	31 d, 1 hr, 35 m	Urinary tract infection	47	Improbable
	41	Y	36 d, 19 hr, 35 m	Complication of original stroke		Improbable
	47	Y	0 d, 2 hr, 25 m	Extension/recurrent ischaemic stroke		Improbable
	53	Y	0 d, 5 hr, 46 m	Extension/recurrent ischaemic stroke		Improbable
RIC	9	Y	0 d, 21 hr, 7 m	Extension/recurrent ischaemic stroke	2	Improbable
	12	Y	0 d, 17 hr, 32 m	Symptomatic Haemorrhagic transformation of infarct		Possible
	16	N	2 d, 6 hr, 40 m	Urinary tract infection		Improbable
	22	Y	0 d, 0 hr, 0 m	Fever, undetermined source	30	Improbable
	39	N	7 d, 10 hr, 10 m	Symptomatic Haemorrhagic transformation of infarct		Possible
	39	N	1 d, 22 hr, 6 m	Lung Malignancy		Improbable
	39	N	21 d, 10 hr, 10 m	Recurrent ischaemic stroke	30	Improbable
	33	N	17 d, 17 hr, 55 m	Pneumonia		Improbable
	42	Y	2 d, 7 hr, 37 m	Urinary tract infection		Improbable
	35	Y	9 d, 20 hr, 5 m	Urinary tract infection	30	Improbable
	52	N	2 d, 23 hr, 23 m	Transient ischaemic attack		Improbable
	44	N	6 d, 19 hr, 55 m	Pneumonia		Improbable

Supplementary Table IV. Serious Adverse Events and Clinical outcomes by thrombolysis

Serious Adverse Event	RIC	Sham	p
Thrombolysed			
Number	16	17	
No with SAE			
Any SAE	5 (31.3)	5 (29.4)	1.0
Fatal	1 (6.2)	3 (17.6)	0.60
All stroke and ND*			
Extension/recurrent ischaemic stroke	1 (6.2)	3 (17.6)	0.60
Symptomatic HTI	1 (6.2)	0	0.49
Early ND	0	1 (5.9)	1.0
Seizure	0	1 (5.9)	1.0
Limb injury	0	0	-
Not thrombolysed			
Number	15	12	
No with SAE			
Any SAE	5 (33.3)	5 (41.7)	1.0
Fatal	1 (6.7)	1 (8.3)	1.0
All stroke and ND*			
Extension/recurrent ischaemic stroke	1 (6.7)	3 (25)	0.29
Symptomatic HTI	1 (6.7)	1 (8.3)	1.0
Early ND	0	0	-
Seizure	0	0	-
Limb injury	0	0	-

Post hoc analyses, performed using 2-sided Fisher's Exact test. Data are number (%)
 SAE, serious adverse event, ND neurological deterioration, HTI haemorrhagic transformation of infarction